ATH-1105, a Small-Molecule Positive Modulator of the HGF/MET System, Is Neuroprotective in Preclinical Models of ALS

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CONCLUSIONS

Treatment with ATH-1105 in vitro resulted in

1. Enhanced MET, AKT, and ERK activation
2. Protection of motor neuron-muscle cocultures from glutamate-mediated toxicity
3. Attenuation of glutamate-mediated toxicity in SOD1^{G93A} spinal motor neurons

KEY TAKEAWAY

This study highlights the neuroprotective attributes of ATH-1105 in preclinical models of ALS and supports further investigation into its therapeutic potential

Acknowledgments

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Disclosures

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Disclaimers

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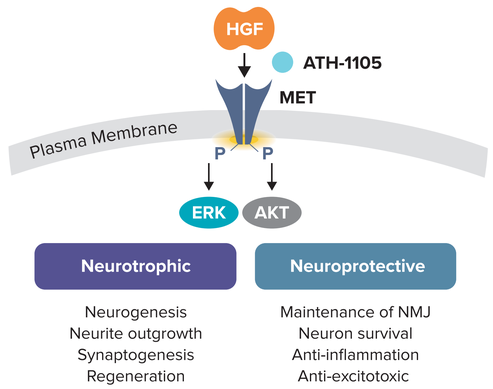
Presented at the ALS Drug Development Summit; May 16-18, 2023; Boston, Massachusetts

INTRODUCTION

Figure 1. Positive modulation of HGF/MET promotes neuroprotective effects through downstream signaling pathways



- ALS pathology is associated with glutamate-mediated toxicity, oxidative stress, mitochondrial dysfunction, axonal degeneration, TDP-43 extranuclear accumulation, NMJ impairment, and motor neuron death[1-3]
  - Extranuclear accumulation of TDP-43 is a pathological hallmark of ALS present in 97% of people with ALS[4]
- Promotion of HGF/MET activity has been reported to have beneficial effects in preclinical models of ALS through its multimodal neuroprotective and neurotrophic actions[2,3,5-7]
- As a positive modulator of the HGF/MET system, ATH-1105 has the potential to alleviate key components of ALS[2,5]

OBJECTIVES

To evaluate the effects of ATH-1105 on glutamate-mediated toxicity in in vitro models of ALS

METHODS

Nerve-muscle coculture impairment assay

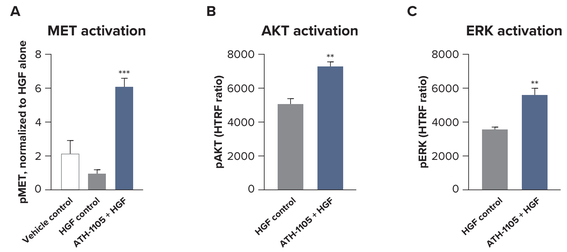
- Whole spinal cord sections, including 4 DRGs, were harvested from E13 Wistar rat embryos and were cocultured on a monolayer of human muscle cells for 27 days, a sufficient culture period to allow formation of functional NMJs
- Mature cocultures were pretreated for 20 minutes with vehicle (DMSO, 0.1%) or ATH-1105 10 nM, 100 nM, or 1 µM and then challenged with glutamate 60 µM for 20 minutes, after which treatment was reapplied for an additional 48 hours
- By use of automatic quantification (Edison Developer; GE Healthcare) of anti–NF-200 immunolabeling of muscle fibers and α-bung labeling of AChRs, cocultures were evaluated to determine motor neuron survival, neurite length, AChR clustering, and the number of motor units

SOD1^{G93A} spinal motor neuron toxicity assay

- Spinal motor neurons were harvested from E14 SOD1^{G93A} rat embryos and cultured for 13 days
  - SOD1^{G93A} is a transgenic model of ALS[8]
- Cultures were pretreated for 15 minutes with vehicle (containing HGF 0.05 ng/mL) or ATH-1105 1 µM and then challenged with glutamate 5 µM for 20 minutes
- After 24 hours of incubation in treatment conditions, immunofloursecence analysis via MetaXpress (Molecular Devices) was used to assess neuronal survival (anti–MAP-2), extranuclear TDP-43 (anti–nuclear-TDP-43), mitochondrial health (MitoTracker), and ER stress (anti-ATF6)

RESULTS

Figure 2. ATH-1105 enhances MET, AKT, and ERK activation in vitro

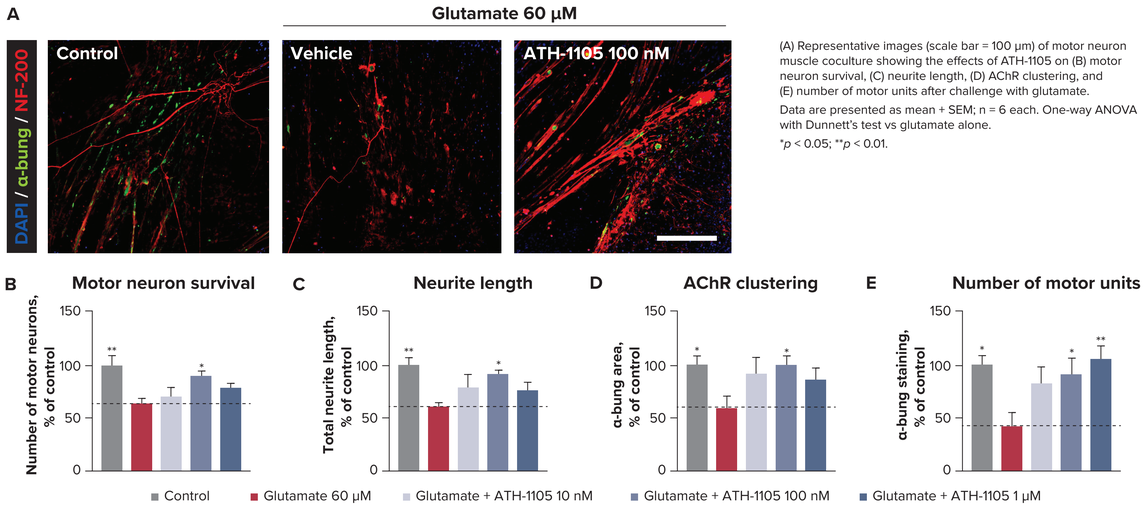


Activation of HGF/MET and its downstream effectors by ATH-1105 in the presence of HGF, as measured by levels of (A) pMET after treatment with ATH-1105 100 pM (one-way ANOVA with Dunnett's test vs HGF alone), (B) pAKT after treatment with ATH-1105 1 µM (unpaired *t* test vs control), and (C) pERK after treatment with ATH-1105 1 µM (unpaired *t* test vs control). Methodological details can be found in the supplemental information (**QR code**).

Data are presented as mean + SEM; n = 3 each.

$^{**}p < 0.01$; $^{***}p < 0.001$.

Figure 3. ATH-1105 protects against glutamate-mediated toxicity in nerve-muscle cocultures

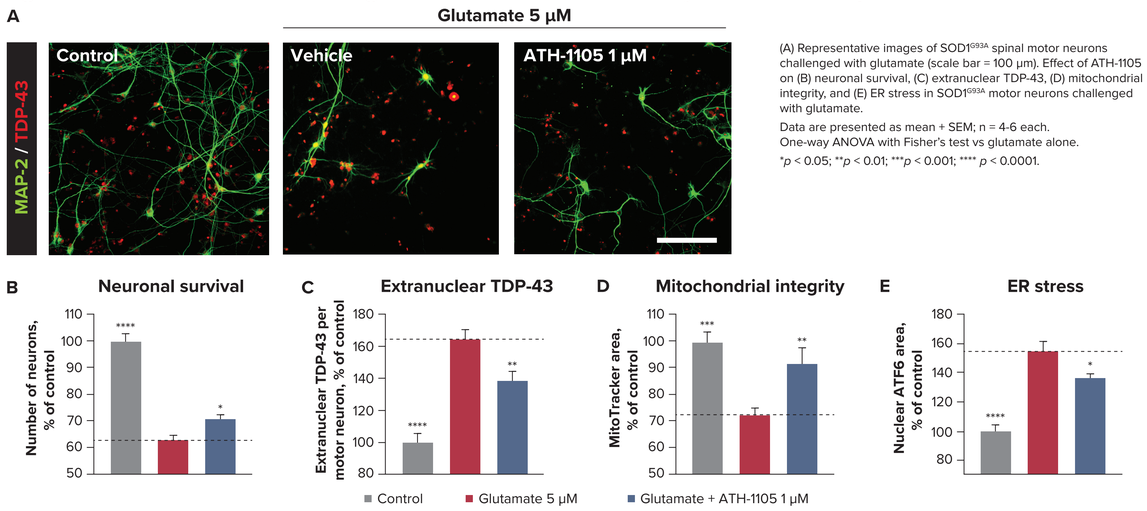


(A) Representative images (scale bar = 100 µm) of motor neuron muscle coculture showing the effects of ATH-1105 on (B) motor neuron survival, (C) neurite length, (D) AChR clustering, and (E) number of motor units after challenge with glutamate.

Data are presented as mean + SEM; n = 6 each. One-way ANOVA with Dunnett's test vs glutamate alone.

$^{*}p < 0.05$; $^{**}p < 0.01$.

Figure 4. ATH-1105 attenuates glutamate-mediated toxicity in SOD1^{G93A} spinal motor neurons



(A) Representative images of SOD1^{G93A} spinal motor neurons challenged with glutamate (scale bar = 100 µm). Effect of ATH-1105 on (B) neuronal survival, (C) extranuclear TDP-43, (D) mitochondrial integrity, and (E) ER stress in SOD1^{G93A} motor neurons challenged with glutamate.

Data are presented as mean + SEM; n = 4-6 each. One-way ANOVA with Fisher's test vs glutamate alone.

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

Abbreviations **α-bung**, α-bungarotoxin; **AChR**, acetylcholine receptor; **AKT**, protein kinase B; **ALS**, amyotrophic lateral sclerosis; **ANOVA**, analysis of variance; **ATF6**, activating transcription factor 6; **DAPI**, 4′,6-diamidino-2-phenylindole; **DMSO**, dimethylsulfoxide; **DRG**, dorsal root ganglia; **ER**, endoplasmic reticulum; **ERK**, extracellular signal–related kinase; **HGF**, hepatocyte growth factor; **HTRF**, homogenous time-resolved fluorescence; **MAP-2**, microtubule-associated protein 2; **NF-200**, neurofilament-200; **NMJ**, neuromuscular junction; **P**, phosphorylation; **pAKT**, phosphorylated AKT; **pERK**, phosphorylated ERK; **pMET**, phosphorylated MET; **SEM**, standard error of the mean; **SOD1^{G93A}**, superoxide dismutase 1 G93A mutation; **TDP-43**, TAR DNA-binding protein 43

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SUPPLEMENTAL INFORMATION

MET activation assay

- HEK293 cells were incubated with vehicle control, HGF 1 ng/mL, or HGF 1 ng/mL plus ATH-1105 100 pM in 6-well plates for 15 minutes
- Cells were lysed, and levels of pMET stimulated by each treatment were measured via ELISA

AKT/ERK activation assay

- HEK293 cells were incubated with vehicle control (containing HGF 2 ng/mL) or ATH-1105 1 µM in 96-well plates for 20 minutes
- Cell lysates were fluorescently immunolabeled using anti-pAKT and anti-pERK antibodies, and quantified using an HTRF reader

Abbreviations: **AKT**, protein kinase B; **ELISA**, enzyme-linked immunosorbent assay; **ERK**, extracellular signal–related kinase; **HEK293**, human embryonic kidney 293; **HGF**, hepatocyte growth factor; **HTRF**, homogenous time-resolved fluorescence; **pAKT**, phosphorylated AKT; **pERK**, phosphorylated ERK; **pMET**, phosphorylated MET.

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